OPOLE SCIENTIFIC SOCIETY
NATURE JOURNAL
No 47 – 2014: 22-30

SHORE FLIES (DIPTERA: EPHYDRIDAE) FEEDING ON BLUE-GREEN ALGAE

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ABSTRACT: Cyanobacterial blooms are a worldwide phenomenon in nearly all kinds of fresh water. Eutrophication and cyanotoxins cause serious ecological problems and human or animal health hazard. 25 known species of the family Ephydridae whose larvae feed on toxic cyanobacteria (Cyanophyceae) were listed. The list of these species was given. General information about adaptations of adults and immature stages to this toxic habitat was presented. Certain instructions about adults and larvae collecting, rearing and methods of stomach content analysis were provided. As a futuristic approach, we consider possibility of using the larvae as potential natural enemies reducing the populations of cyanobacteria.

KEY WORDS: Ephydridae, cyanobacteria, adaptations, collecting and rearing techniques.

Introduction

The Ephydridae, or shore flies, is a family belonging to acalyptrate Diptera. The taxon is comparatively large and consists of at least 1920 species occurring nearly all over the world (Mathis and Zatwarnicki 1995, with update). The flies are closely associated with wetlands or

1 Izabela Ryczko is a recipient of a Ph. D. scholarship under a project founded by the European Social Found.

moist environments. Adults and immature stages have broad ecological and trophic specializations. Adults inhabit areas with fresh or muddy water as marshes, swamps, lakes, ponds, streams, seepages and sea-shores. Most of larvae live in the following habitats: mud, grass and sand shores, floating vegetation and algal mats, detritus deposits and wet meadows. In addition to exposed wet sediment they live within stems, shoots, roots and leaves of aquatic plants (Zatwarnicki 1997).

Species of Ephydridae feeding on cyanobacteria are able to exist in inhospitable habitats as hot springs and highly alkaline or saline water where occurrence of other organisms are drastically limited. It is mainly due to lethal influence of extreme and harmful biophysical conditions as temperature, salinity, pH and mineral or organic pollution (Foote 1977, Zatwarnicki 1997, Krivosheina 2008). This indicates the evolutionary success of this dipteran family (Wirth and Mathis 1979).

In this paper authors present data about shore flies feeding on cyanobacteria with their morphological adaptations which allow them to live and feed on toxic blue-green algae. Some information about collecting, rearing of individuals and methods of stomach contents analysis are elucidated. The potential usage of shore flies to limit massive algal blooms are also discussed.

The importance of blue-green algae and its toxicity

Cyanobacteria, called also blue–green algae are autotrophic prokaryotes. Probably the oldest organisms on the Earth; their fossils date to 3.5 billion years old (Krivosheina 2008). Blue–green algae are characterized by diversified colour, depending on concentration of the pigment (Pliński and Komárek 2007).

It is believed that success of blue–green algae in adapting to modern habitats related to their long evolutionary history. They are resistant to many hostile environmental conditions like low oxygen level, desiccation, influence of ultraviolet – B and C – radiation and have wide temperature range tolerance (- 83^{o}C to 90^{o}C) (Whitton and Potts 2000). In terms of global biomass they are the most important organism on the Earth (3 x 10^{14} g C) (Whitton and Potts 2012).

25 to 75% of cyanobacterial blooms are toxic. Both extracellular and intracellular products of cyanobacteria may be lethal. Released cyanotoxins can lead to human and animal health hazards or even death (Chorus 2001, Bláhová et al. 2008). Following cyanotoxins are released by particular genera of cyanobacteria: hepatotoxin: *Anabaena*, *Nostoc*, *Hapalosiphon*; neurotoxin: *Anabaena*, *Oscillatoria*, *Cylindrospermum*, *Lyngbya*;

dermatotoxin: *Lyngbya*, *Oscillatoria.* Cyanobacteria affect also organisms which try to ingest them (Bláha et al. 2009). Blue-green algae are seldom utilized but some herbivore grazers (Bláhová et al. 2008, Krivosheina 2008). Nevertheless, the largest number of examples of successful feeding on cyanobacteria is known among ephydrids (Tab. 1). Coexistence between ephydrids and cyanobacteria in unfavorable habitats seemingly allow them to avoid predation and achieve evolutionary success.

Shore flies adaptations to live and feed on blue-green algae

The adults of Ephydridae feeding on cyanobacteria were seldom studied. However, some authors have observed their occasional incidence of consuming algal mats (Tab. 1). Some specimens may have elongated tarsi and straight claws which are adaptations to move over the algal surface (Foote 1982). In natural environments adults have applied their mouthparts to the surfaces of *Cylindrospermum* (Foote 1981). The shape of adult proboscis are quite broad, fleshy and boot-shaped. Thus, the flies are basically algal feeders and can efficiently gather microorganisms from flat, open surfaces (Simpson 1976). Nevertheless, the morphology of the adult mouthparts have not been studied in detail. Additionally, Ephydridae may be present in a large amount on blue-green algal mats because of strategy of oviposition. Females insert eggs into the mats what makes them unavailable for predators (Wirth and Mathis 1979).

Foote (1979) divides larvae species into three trophic groups: polyphagous, oligophagous and species which are specific in their trophic ecology (monophagous). The list of species digesting cyanobacteria are summarized in the Tab. 1. Species from the tribes *Ephydrini* and *Scatellini* are trophic generalists, in contrast to the tribe *Hyadinini*, which are trophic specialists, ecologically unified by preference for the floating algal-mat habitat. Thus, members of *Hyadinini* coexist in the same habitat can be ecologically isolated by utilizing different food resources e.g. *Nostima approximata* prefer *Oscillatoria* as a food while *Pelina truncatula* and *Lytogaster excavata* grazing on *Cylindrospermum* (Foote 1977, 1979, 1983, Connell and Scheiring 1981).

Ecological limitations in water basins with algal blooms result in appearance of special adaptations in ephydrids. Larvae are equipped with following morphological adaptations allowing them to exist within algal mats. Pseudocephalic segment with apicoventrally sensory plates and ventrally facial mask. The facial mask has distinguish mouthhooks cooperating with 4 rows of comblike structures placed on either side of oral aperture. Each of the rows are provided with 7 – 10 tapering teeth along posterior margin (Foote 1981a). These elements of mouthparts are responsible for raking across the algal matrix, shredding the algal mats and ingesting whole trichomes or theirs fragments. The pharyngeal sclerite bears nine flat-topped ridges in the bottom of the pharynx (absent in *N. approximata*) (Foote 1981, 1981a, 1982, 1983). Some species showing flickering motions are filter feeders e.g. *Scatella stagnalis* (Foote 1981a, 1993).

The type of food ingesting depends on the larval instar. Small unicells are preferred by newly hatched larvae while older specimens have a broader spectrum of utilization blue-green algae, mostly their trichomes (Mathis 1982). During movements across the algal substrates they left noticeable track with absence of algae (Foote 1993). Depending on species larvae can feed on the surface of the algal colony e.g. *L. excavata* (Foote 1981a) or submerged e.g. *Setacera atrovirens* (Foote 1982). Larvae from the same species usually fed together on the algal surface and none of antagonistic behaviour was observed (Foote 1981, 1981a, 1983, 1993).

Some of species are provided with crochet-bearing prolegs with 2 or 3 rows of hooks, which allow them to attach and move through the algae (Oscoz et al. 2011). Respiration is possible through the respiratory tube, which remains in contact with the atmosphere while the larvae is hanging down from the surface. Some specimens of larvae return to the water surface for respiration. These adaptations give them the ability to feed completely submerged. Prior to formation of the puparia larvae decrease food consumption and empty the gut contents. Thereafter leave the algal mats and start to move to the drier part of the substrate or attach to narrow leaves or stems using the last prolegs (Foote 1993). Formation of the puparia is often below the water surface. That indicates that submergence has no effect on further development (Simpson 1976, Collins 1977, Mathis 1982). The mature larvae and puparium several of presented species were described and illustrated: *Scatella* (Zack and Foote 1978), *Pelina* (Foote 1981), *Lytogaster* (Foote 1981a), *Setacera* (Foote 1982), *Nostima* (Foote 1983).

Collecting

Adults can be collected using a hand net. To determinate species and sex of the flies should be temporarily immobilized with ethyl acetate, ether or carbon dioxide (Zatwarnicki 1997).

An efficient method for obtaining immature stages is collecting suspected larval habitat to the plastic box. The box with samples should be placed under fluorescent lighting and aerated. In the next stage it should be checked for larvae and puparia. Another useful method is floating, which is stirring up collected earlier substrate and searching the water surface for floating immature stages. This method can be extended by rinsing small portion of substrate in a kitchen strainer and searching for larvae and puparia (Mathis and Simpson 1981).

Another method is Tullgren funnel, also known as Berlese funnel or Berlese trap. It is based on positive geotaxis in response to gradually decreasing humidity. Temperature gradient is used for drying habitat's sample, in result the hygrophilous animals lead on to the preservative fluid (Gordh and Headrick 2011).

Rearing techniques

The collected specimens of one species should be placed in breeding chambers. They can be prepared from glass containers with a capacity of approximately 100 ml. To form a glass cylinder the bottom of a glass container was removed, then covered by piece of nylon and secured through rubber band. The other part of breeding chamber was placed in a petri dish which should be filled with 1.5% agar medium. Additionally on the petri dish should be placed suitable medium for the species of blue-green algae as a food source. When desiccation or depleted will be observed petri dishes should be replaced.

In each glass cylinder should be placed at least one female and one male. Some of them can contain from 2 to 14 specimens in different sex ratios. After oviposition, the eggs should be removed to different petri dish with same algal composition by camel hair brush. After pupation adults should be removed from breeding chambers (Connell and Scheiring 1981). The generation can last from two weeks to a month and half, adults can be sustained for a whole year (Oscoz et al. 2011).

Analysis of stomach content

We can distinguished two methods of stomach content analysis. First, the gut should be removed, it contents should be placed in a temporary tap-water to examine for the presence of algae and diatoms. Then the sample should be placed in a drop of 20% hydrogen peroxide and heated slowly for 10 minutes, to clear remaining diatoms. Then to permanently mount the sample it should be placed in Hyrax medium. The second method is washing specimens in a detergent solution and digesting them in a hot solution of 20% nitric acid. After this process the sample should be cooled and then the acid should be removed from it. The remaining undigested stomach contents should be permanently mounted in Hyrax medium (Deonier 1972).

Conclusions

As yet there is lack of informations about wide range of reduction of cyanobacterial blooms by ephydrid species. However, there was suggested that ability of utilization of blue-green algae by ephydrids larvae may have considerable practical and ecological significance. It is particularly in case of trophic specialist (Foote 1977, 1981). Larval burrowing cause mechanical damage of the algal mats and accelerate theirs disintegration and lead to decomposition (Foote 1982). During the short winter days algal mats can be consume by *Paracoenia* and *Ephydra* faster than it can be replaced (Wirth and Mathis 1979). Larvae of *S. atrovirens* were found to be utilizing extensively large colonies of *Nostoc* (Foote 1977). This suggests that this group of flies can ingest, digest and assimilate blue-green algae and overcome the algal defense mechanisms such as toxins release (Foote 1977).

Thus, in our opinion Ephydrids should be considered as organisms with potential ability to reduce blue–green algae. They occur worldwide, many species are cosmopolitan and well adapted to exist within cyanobacterial mats. Apart from larva stadium, some adults can also feed on blue-green algae. Moreover, further research is vital due to lack of knowledge about physiological mechanisms which allow them to feed on cyanobacteria.

Acknowledgements

The authors thank to Professor Tadeusz Zatwarnicki for valuable comments.

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PRZYWÓDKI (DIPTERA: EPHYDRIDAE) ODŻYWIAJĄCE SIĘ SINICAMI

W pracy przedstawiono przegląd gatunków muchówek Ephydridae żywiących się toksycznymi sinicami. Częste współwystępowanie tych organizmów w środowisku sugeruje możliwość wykorzystania tych muchówek do walki z toksycznymi zakwitami. Przedstawiono szereg informacji z zakresu adaptacji do tego specyficznego środowiska i pokarmu. Załączono tabelę zawierającą zestawienie 25 gatunków Ephydridae, żywiących się sinicami produkującymi toksyny. Opisano metody służące do połowu i hodowli muchówek oraz metody pozwalające wyizolować ich zawartość przewodu pokarmowego.

Tab. 1. Ephydridae feeding on blue-green algae

Subfamily	Tribe	Species of ephydrid	Stage of development	Feeding habits	Cyanobacteria	Sources
Ilytheinae	Hyadinini	*Pelina truncatula*	larvae	oligophagous	*Lyngbya spiralis, Anabaena variabilis, Anabaena sp., Cylindrospermum sp., Oscillatoria tenuis, Oscillatoria sp.*	Foote 1977, 1979, 1981, 1983
		Nostima approximata	larvae /adults	oligophagous	*Anabaena variabilis, Oscillatoria limosa, Oscillatoria tenuis, Symploca muscorum*	Foote 1983
		Lytogaster excavata	larvae/ adults	oligophagous	*Anabaena variabilis, Anabaena sp., Cylindrospermum sp., Lyngbya sp., Phormidium sp.*	Foote 1977, 1979, 1981a, 1983
		Lytogaster abdominalis	larvae	monophagous	*Cylindrospermum sp.*	Foote 1977
		Lytogaster flavipes	larvae	monophagous	*Cylindrospermum sp.*	Foote 1977
		Lytogaster furva	larvae	monophagous	*Cylindrospermum sp.*	Foote 1977
		Hyadina binotata	larvae	oligophagous	*Anabaena sp., Cylindrospermum sp., Lyngbya sp., Phormidium sp.*	Foote 1977, 1979
		Hyadina subnitida	larvae	oligophagous	*Anabaena sp,. Anacystis sp., Cylindrospermum sp., Lyngbya sp., Phormidium sp.*	Foote 1977, 1979
		Hyadina neglecta	larvae	monophagous	*Cylindrospermum sp.*	Foote 1977
		Hyadina albovenosa	larvae /adults	oligophagous	*Anabaena variabilis, Anabaena flos-aquae, Lyngbya spiralis, Nostoc commune,Calothrix sp., Cylindrospermum sp., Oscillatoria tenuis, Spirulina sp., Symploca muscorum, Synechococcus leopoliensis*	Foote 1993
		Axysta cesta	larvae	monphagous	*Lyngbya sp.*	Foote 1977
Ephydrinae	Ephydrini	*Ephydra riparia*	larvae	oligophagous	*Anabaena sp., Gloeocapsa sp.*	Foote 1979
		Ephydra cinerea	larvae / adults	polyphagous	*Nostoc sp., Aphanothece utahensis, Microcystis packardii*	Collins 1979, Deonier 1972, 1993
		Setacera pacifica	larvae	polyphagous	*Nostoc sp., Anabaena sp.*	Foote 1977, 1979
		Setacera atrovirens	larvae / adults	polyphagous	*Anabaena variabilis, Anabaena sp., Cylindrospermum sp., Lyngbya sp., Nostoc commune, Nostoc sp., Oscillatoria tenuis, Oscillatoria chalybea, Oscillatoria sp., Phormidium sp.*	Foote 1977, 1979, 1982
	Scatellini	*Scatella stagnalis*	larvae / adults	polyphagous	*Anabaena flos-aquae, Gloeocapsa sp., Nostoc commune, Nostoc muscorum, Nostoc sp., Cylindrospermum sp.*	Foote 1977, 1979,1978
		Scatella picea	larvae	polyphagous	*Lyngbya sp.*	Connell 1981, Foote 1979
		Scatella thermarum	larvae / adults	?	*Phormidium laminosum, Mastigocladus laminosus, Hapalosiphon laminosus,*	Deonier 1972, Wirth and Mathis 1979
		Coenia curvicauda	larvae	polyphagous	*Anabaena flos-aquae, Anabaena variabilis, Calothrix sp., Oscillatoria tenuis, Synechococcus leopoliensis*	Foote 1990
		Paracoenia bisetosa	larvae	?	*Phormidium sp., Mastigocladus sp., Oscillatoria sp.*	Mathis1975, Wirth and Mathis 1979
		Paracoenia turbida	larvae / adults	?	*Phormidium sp., Mastigocladus sp., Oscillatoria sp.*	Deonier 1972, Mathis1975, Wirth and Mathis 1979
		Paracoenia beckeri	larvae	?	*Phormidium sp., Mastigocladus sp., Oscillatoria sp.*	Wirth and Mathis 1979
		Paracoenia calida	larvae	?	*Phormidium sp., Mastigocladus sp., Oscillatoria sp.*	Mathis1975, Wirth and Mathis 1979
		Paracoenia wirthi	larvae	?	*Phormidium sp., Mastigocladus sp., Oscillatoria sp.*	Mathis1975, Wirth and Mathis 1979
		Paracoenia platypelta	larvae	?	*Phormidium sp., Mastigocladus sp., Oscillatoria sp.*	Mathis1975, Wirth and Mathis 1979